MICROSCOPIC OBSERVATIONS OF GRAPE SKINS TREATED IN PULSED ELECTRIC AND HIGH FREQUENCY ELECTROMAGNETIC FIELDS

Laslo Vasile*, Bandici Livia **, Bandici Gheorghe *, Vicas Simona *

*University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru Street, 410048, Oradea, Romania; e-mail: vasilelaslo@yahoo.com
**University of Oradea, Department of Electrical Engineering, Faculty of Electrical Engineering and Information Technology, 1 Universitatii Street, 410087 Oradea, Romania, e-mail: lbandici@uoradea.ro

Abstract

Nowadays we try to find new solutions to improve traditional methods with the aim of obtaining, on the one hand, high quality products, rich in bioactive principles, and on the other hand, to have reasonable costs and to protect the environment. In the wine industry, "green technologies", such as microwave and pulsed electric field treatments, have been introduced to reduce fermentation time, the amount of SO_2, and to obtain wines that are rich in bioactive compounds. The aim of this paper was to treat the grape mash from the Pinot Noir variety, both in MW and PEF, and to highlight the microscopic changes that occur at cell level. In addition, from the mash of the treated grapes we also determined the total monomeric anthocyanin pigment content to highlight the efficiency of the extraction compared to the control sample that remained untreated. The macroscopic observations show changes in the cell membranes that resulted in the mass transfer, and so the content of bioactive compounds of interest is higher.

Key words: Pulsed Electric Field, High Frequency Electromagnetic Field, grapes, Total monomeric anthocyanin pigment

INTRODUCTION

Wine quality depends largely on the manufacturing technology. The flowchart of grape processing is determined by the type of wine that is to be obtained, by complying with the specific oenological requirements, and by economic factors.

Anthocyanins are a group of secondary metabolites responsible for the color of fruit, vegetables, and flowers. Anthocyanins are the main polyphenols present especially in the skins of red grapes.

Also, these bioactive compounds have various beneficial effects on human health, such as reducing the incidence of coronary heart diseases, improving visual acuity, maintaining normal vascular activity, as well as anticancer, antimutagenic, anti-inflammatory, and antioxidant effects (Bagchi et al., 2000; Chacona et al., 2009; Xia et al., 2010). In the wine industry, anthocyanins play an important part in the quality of red wine.

In recent years, innovative technologies, such as ultrasounds, microwaves (HFEF), and pulsed electric field (PEF) have been tested in the winemaking industry for energy efficiency (Toepfl, et al., 2006), but also to obtain wines with a higher content of bioactive compounds thus improving wine quality (Vilkhu, et al., 2008; Clodoveo et al., 2016).

Electromagnetic methods promise to be the most effective procedures to improve solid-liquid separation. Injecting a direct current has been studied in literature, leading to the conclusion of an increased efficiency due to the combination between the effect of pressure and electroosmosis. Identifying technological opportunities whose usefulness has been demonstrated in various branches of scientific knowledge to improve grape processing, must conditioning, and winemaking processes.

Applying an alternative voltage of different shapes (most often pulsed) remediates this disadvantage and has the effect of breaking the cell, a phenomenon called electroplasmolysis. It is expected that the efficiency of applying PEF depend on the type of cell, pulse amplitude, pulse duration, the number of pulses etc.

Preliminary results show that the use of electric fields in processing the mash has demonstrated the prospects of this technique to obtain optimal results by increasing the yield of the must, increasing extractivity, and reducing must viscosity. Applying PEF to grape and must processing ensures the possibility of preserving some appreciable qualities of valuable compounds, such as polyphenols, flavonoids, catechins, and high values with a beneficial effect of antiradical and antioxidant power, in the wine obtained, elements that configure the valuable profile of the wine.

The aim of this paper was to treat the grape mash derived from the Pinot Noir variety, both in HFEF and PEF, and to highlight the microscopic changes that occur at cell level. In addition, from the mash of the treated grapes we also determined the total monomeric anthocyanin pigment content to highlight the efficiency of the extraction compared to the control sample that remained untreated.

MATERIAL AND METHOD

Research activities have been conducted in the Laboratory of Biotechnology (microscopic observations), the Laboratory of Oenology (PEF and HFEF treatments of the grape mash), and the Laboratory of Chemistry and Biochemistry (spectrophotometric determination of anthocyanins), laboratory that is also registered on the ERRIS platform (https://erris.gov.ro) of the Faculty of Environmental Protection of the University of Oradea.

Grape variety and PEF and HFEF treatments

The Pinot Noir grapes have been harvested in the Crişana-Sântimreu vineyard, in the year 2016. During the optimal ripeness stage it has a Brix index of 23^0 Brix and total acidity of 5.8 g / L. After the declustering and crushing of the grapes, the samples have been treated in pulsed electric field (PEF) and high frequency electromagnetic field (HFEF). From each treatment were taken samples both for microscopic observations and for determining the anthocyanin content in the must.

To treat grape mash (consisting of skins, seeds, and pulp) in HFEF we used a power between 100 and 1000 W. The temperature during processing did not exceed 70^0 C in order to obtain a high quality end product, in which the compounds of interest should not be destroyed.

To treat the mash in PEF, a system of two pairs of two conductor drums that rotate in opposite directions and that train the grape mash was used. Pulses of 150 µs of 7kV/cm were applied with a frequency of 178 Hz.

Microscopic observations

We used the conventional microscopic technique (light microscopy) to highlight the structural changes resulting from the application of PEF and HFEF to the grape mash. We made thin cross sections that were placed on the microscope slide and viewed using a Hund Wetzlar microscope.

Total monomeric anthocyanin pigment content (MAP)

Total monomeric anthocyanin pigment content (MAP) was determined by the pH-differential method (Giusti & Wrolstad, 2001). Two dilutions of each sample were performed, one in phosphate buffer KCl 0.025M (pH=1) and another one in acetate buffer 0.4M (pH=4.5). The samples were maintained 20 minutes for balancing, after which their absorbances at λmax and 700 nm were read. The absorbance of the diluted samples was calculated according to the formula (1).

$$\mathbf{A = (A\ \lambda_{max} - A_{700})pH_{\ 1.0} - (A\ \lambda_{max} - A_{700})pH_{\ 4.5}} \qquad (1)$$

The MAP concentration in the must and wine samples was calculated using the formula (2).

$$\mathbf{MAP\ (mg/liter) = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)} \qquad (2)$$

where MW is the molecular weight, DF is the dilution factor, and ε is the molar absorptivity. The results were reported compared to cyanidin-3-glucoside (MW = 449.2 and ε= 26,900).

Statistical Data

All the data were processed by one-way analysis of variance (ANOVA) (P = 0.05). Mean value differences were analyzed with Tukey's test (P = 0.05).

RESULTS AND DISCUSSION

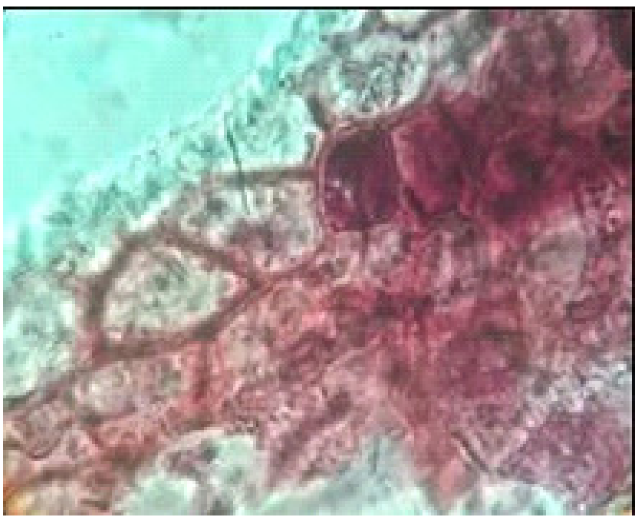
Grape skin represents approximately 5-10% of the total dry weight of the grape and acts as a hydrophobic barrier to protect the fruit from physiological and climatic injuries, dehydration, fungal infections and UV-C light (Fava et al., 2011). The epidermal cell wall can be distinguished from other cell walls of plants due to the presence of a thick lipid layer (cuticular membrane) stored in outermost regions. Generally, there are five layers, from the outside to the inside:

1. The epicuticular wax layer (amorphous, crystalline, or semi-crystalline);
2. The cuticle proper (consisting only of cutin);
3. The cutinized layer (mainly composed of a polysacharride matrix, cutin, and intracuticular waxes). The cuticle proper and the cutinized layer constitute the cuticular membrane;
4. The pectin layer, generally composed of pectin polysacharrides;
5. The cellulosic layer which is a strong network of cellulose microfibrils linked by hydrogen bonding to xyloglucans, pectin polysacharrides and minor cell wall constituents (structural proteins, enzymatic proteins, hydrophobic compounds and inorganic molecules).

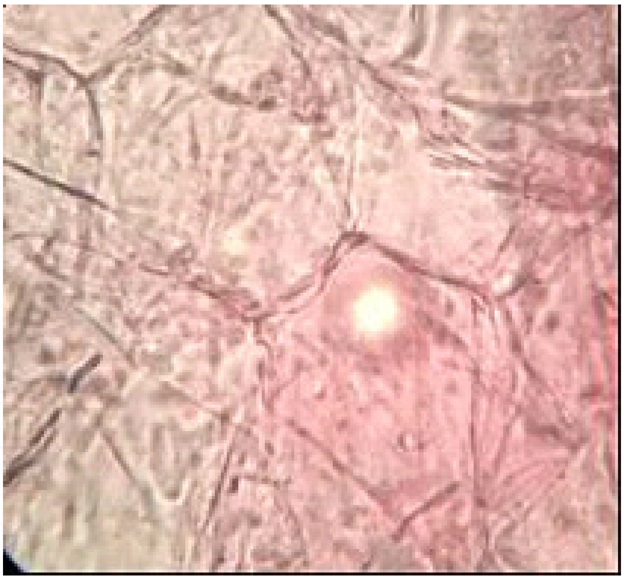
After the epidermal cell wall comes the hypoderm that includes phenolic compoundws and consists of 3-4 layers (Fava et al., 2011).

Of the bioactive compounds that are responsible for the color of grapes, the anthocyanins are present in the skin of the grapes. There is a close link between the color of the grape and the anthocyanin profile.

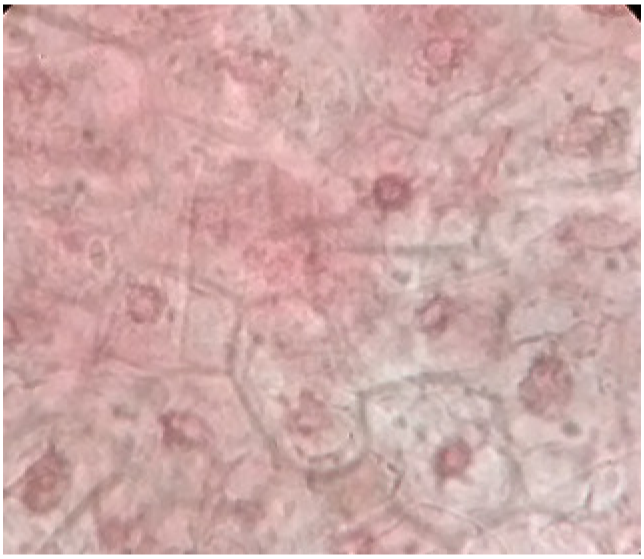
The microscopic images obtained after PEF and HFEF treatments of grape skins, as well as the control sample are shown in Figure 1 a, b, c.



a)



b)



c)

Fig. 1. Microscopic images of grape skins. a. Untreated grape skin (control sample).
b. PEF treated grape skin. c. HFEF treated grape skin

In figure 1a, in the case of the untreated grape skin, we notice well defined cells, and the presence of anthocyanins in the cell vacuoles well defined from the rest of the cells. The use of HFEF in grape processing in order to obtain wine results in reduced winemaking time and improves its quality (Clodoveo et al., 2016).

In Figure 1c. we observe that, after HFEF treatment, the cell membrane is thinned, and the anthocyanin content is dispersed across the cell mass. Carew et al., 2013 have demonstrated that microwave treatment of Pinot Noir grapes has determined a rapid extraction of polyphenols compared to the control sample, and also aromatic compounds were present in a larger amount. This was due to the fact that, after microwave treatment, the thermal inactivation of the degradation enzymes of the flavors took place. PEF treatment involves applying a high intensity (20-80 kV) pulsed electric field, of very short duration (microseconds) in the case of a liquid vegetal material placed in a treatment chamber where there are two electrodes.

Applying this treatment to the grape mash results in the temporary or permanent formation of pores in the cell membrane, a phenomenon that depends on the electric field intensity, the duration and the number of pulses (Clodoveo et al. 2016). This electroporation leads to the transfer of low molecular weight compounds, followed by the disintegration of the cell membrane (De Vito et al, 2008). In Figure 1b. we observe ruptures in the cell membrane, and the anthocyanins are not found in vacuoles with well defined membranes.

After the treatment of the mash, the samples have been centrifuged, then the MAP content has been determined. The results showed that, after PEF treatment, the highest MAP content has been reached compared to the control sample, about 1.5 times higher (Figure 2).

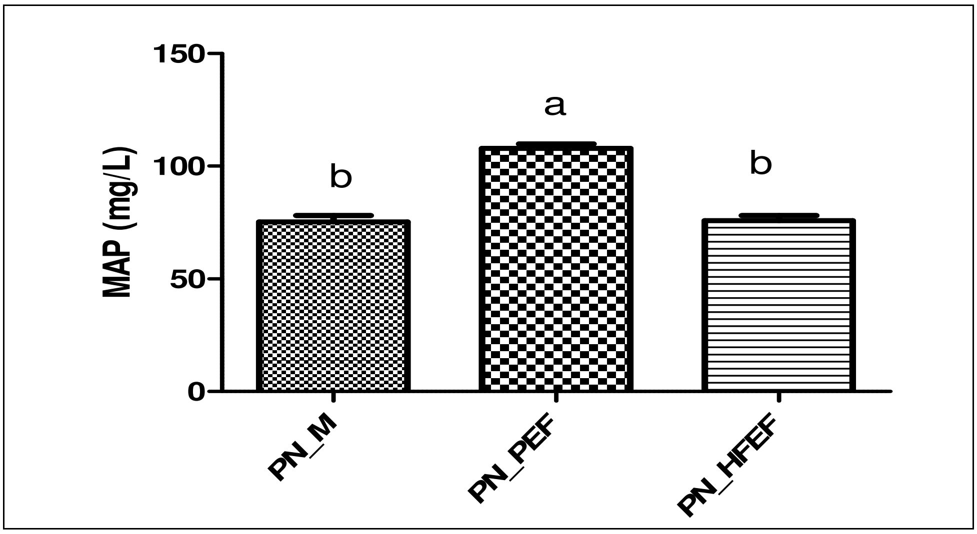


Fig. 2. The parameter values are displayed as mean ±standard deviation (n=6). The different letters prescribe statistical significant differences between the samples (P = 0.05)

In contrast, HFEF treatment did not lead to a significant increase in MAP compounds compared to the control sample (75.85 mg/L, and 75.35 mg/L, respectively) (Figure 2). HFEF treatment results in a good extraction due to the synergistic effect between heat and the mass gradient (Clodoveo et al., 2016).

Bandici et al., 2016, investigated the effect of high frequency electromagnetic field applied at the first step of wine technology, on the total polyphenols content and antioxidant capacity of wines. The authors have used Muscat Ottonel, Merlot, and Pinot Noir grapes, harvested in the Crisana-Santimreu vineyard in 2014 (North-West of Romania). The grapes were declustered and crushed, resulting in the grape mash, which has been divided into two parts. A part was treated in high frequency field (HFEF), while the other one remained untreated. Applying HFEF treatment to Pinot Noir grapes resulted in an increase in polyphenols of 217.35% in the mash, while, in the wine, polyphenols increased 64.36 % compared to the control sample (Bandici et al., 2016).

CONCLUSION

Applying innovative technologies (HFEF, PEF) in the winemaking process represents a current approach with many advantages, both economically and in particular in terms of obtaining high quality products, rich in bioactive principles with beneficial effects on human health.

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